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# INTERLEUKIN-34 LEVEL IN GINGIVITIS PATIENTS; CLINICAL AND BIOCHEMICAL STUDY

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## *ABSTRACT*

**Objective:** The objective of this clinical trial was to compare changes in IL-34 level in Gingival crevicular fluid (GCF) before and after nonsurgical periodontal therapy (NSPT) in individuals with biofilm-induced gingivitis in relation to periodontal clinical signs.

**Methods and materials:** Twenty patients, both genders, aged 20 to 50, have been diagnosed with biofilm-induced gingivitis. The following parameters were considered clinically at baseline and three months after NSPT: probing depth (PD), gingival index (GI), and plaque index (PI).

**Results:** 3 months postoperatively, substantial reduction in the level of IL-34 in GCF was detected along with better improvement in all clinical parameters compared to preoperative level in addition to positive correlation among changes in level of GCF IL-34 along with changes in all clinical parameters.

**Conclusion:** IL-34 can be regarded as one of the pro-inflammatory markers of periodontal disease and may be investigated in the future as a potential target for periodontal disease therapy.

**KEYWORDS:** Interleukin-34, biofilm- induced gingivitis, nonsurgical periodontal therapy, Egypt.

## INTRODUCTION

Gingivitis, the mildest form of periodontal disease, may affect up to 90% of the population. Development of supra- and sub gingival plaque, calculus, and gingival irritation are the clinical symptoms of periodontal disease at this stage. There is no apical displacement of junctional epithelium as this displacement occurs in periodontitis. [1]

Biofilm is the principal cause of most gingivitis cases. It is a sticky film that can develop in the gingival sulcus and contains large number of bacteria. Failure of regular removal can cause it

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to harden into calculus, which in turn can irritate the gingiva and lead to tooth loss. *Streptococcus, Fusobacterium, Actinomyces, Veillonella,* and *Treponema* are the most prevalent causative agents of gingivitis.[2]

Some local and systemic etiologic variables increase the risk of dysbiotic microbiome changes in some patients by stimulating various immunoinflammatory responses and by extension, the severity of the disease in those persons is increased. These variables include smoking, diabetes, obesity and iatrogenic factors. [3]

Cytokines are the primary connectors between tissue cells, lymphocytes and other accessory cell populations at barrier sites, which are the first line of defense against pathogens and stimuli. During the active stages of periodontitis, many different cell types, such as fibroblasts, macrophages, and lymphocytes, are responsible for the release of cytokines.[4]. Interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-12 (IL-12), and Tumor necrosis factor-alpha (TNF-α) are all examples of pro-inflammatory cytokines; interleukin-4 (IL-4), interleukin-1 receptor antagonist (IL-1RA), and interleukin-10 (IL-10) are examples of anti-inflammatory cytokines.[5]

Probing depths (PD), bleeding on probing (BOP), clinical attachment level (CAL), plaque index (PI), and radiographs of the alveolar bone are some of the traditional clinical periodontal diagnostic methods. They will be only able to provide information about history of the disease not its current activity.[6]

Recent methods for the identification of oral and periodontal disease could be done using objective tools such as biomarkers which are able to detect and diagnose periodontal disease at different molecular, cellular, tissue, and clinical levels.[7]

Biomarkers are "cellular, biochemical, molecular, or genetic alterations by which a normal, abnormal, or simply biologic process can be recognized or monitored."[8]. They would either take part in the development of the disease itself or be released when the tissues are damaged to detect severity and progression of the disease.[9]

GCF is the most used media for diagnosis of periodontal condition due to its site-specific feature which offer the foundation for case-specific diagnostic tests for periodontal disease. Additionally, it is well-liked used because it is simple to operate, long-lasting, and inexpensive. In addition to GCF, saliva, serum, sub gingival plaque, and tissue biopsies are all potential sources of periodontal disease biomarkers.[10]

Macrophage colony-stimulating factor, also known as CSF-1, is a cytokine that plays a significant role in regulating the survival, proliferation, and differentiation of mononuclear phagocyte cells. These cells include monocytes, macrophages, and osteoclasts. CSF-1 is produced by endothelial cells, osteoblasts, and fibroblasts and it interact with the colony-stimulating factor receptor (CSF-1R) (c-FMS) [11]

Another active component of CSF-1R was discovered known as IL-34 in 2008. The mRNA for IL-34 is expressed in a wide variety of tissues and organs, including the colon, prostate, small intestine, thymus, heart, spleen, nerves and ovaries [12]

It is crucial to understand the role of IL-34 in disease in order to detect its mode of action. Its expression is linked to the severity and progression of disease in some conditions, such as autoimmune disorders, infections (both viral and bacterial), inflammation, and cancer; however, its role in periodontal disease is not fully understood.[13]

The objective of this research was to investigate the changes in GCF levels of IL-34 pre & post non-surgical periodontal therapy (NSPT) and to correlates these changes with changes in clinical parameters.

## SUBJECT AND METHODS

### Clinical trial design & sample size calculation

There was a total of twenty cases. The Department of Oral Medicine, Periodontology, and Oral Diagnosis at Ain Shams University with the approval of Ethics Committee (FDASU-Rec IM112108) provided patients for this study. All of the participants were informed about the aim of this clinical trial, then they filled out an informed consent form.

Inclusion and exclusion criteria for the cases were as follows: Patients diagnosed with biofilm-induced gingivitis. They were not suffering from any medical condition that could affect the treatment's outcome and this was done using (**Cornell Medical Index-Health Questionnaire**). Superior adherence to plaque-controlling instructions & ease of access to program check-up. The following characteristics excluded a candidate from further consideration such as pregnancy, smoking, lactation and disabled individuals.

### Treatment Protocol:

Prior to the beginning of this clinical trial, detailed case history and full mouth examination was performed on all of the patients who were eligible to participate in the study. At the baseline and three months after NSPT, PD, GI, and PI were measured from deepest selected sites using the **University of Michigan O-probe with William's graduation.**

**GCF Sampling:** Prior to taking samples, neighboring teeth were isolated using cotton rolls in order to ensure accurate results. GCF was collected on paper strips (Periopaper, Ora Flow Inc., Amityville, New York, USA) that were put into the periodontal pocket until resistance was felt and then remained for 30 seconds. This was done so that site selection could be standardized and an appropriate sample amount could be obtained.[14] The samples were collected immediately and three months after therapy. Samples that included traces of saliva contamination were thrown away. Until the results of the research could be interpreted, each sample was kept in an Eppendorf tube at a temperature of -20 degrees Celsius.[15]

Ultrasonic scaling with a DTE D5 LED Ultrasonic Scaler (Guilin Woodpecker Medical Instrument Co., LTD. China) was used to perform NSPT on all cases involved in the study.

**As part of the postoperative care**: each case was given specific instructions on how to practice meticulous oral hygiene, which included brushing their teeth with a medium-sized toothbrush & regular tooth paste at least three times per day, rinsing their mouths two to three times per day with warm saline, and using dental floss or an interdental brush.

**Postoperative evaluation & assessment:** Recalling the cases back after three months for a follow-up appointment and were asked to fill 'the Patient Satisfaction Questionnaire Short Form (PSQ-18) to evaluate their satisfaction about the therapy. [16]

The levels of IL-34 in GCF samples were determined using a commercially available method called Enzyme-Linked Immunosorbent Assay (ELISA), which was supplied by Bioassay Technology Laboratory (Shanghai, China) with Cat. No. E5523Hu. This was done in line with the instructions provided by the manufacturer.

**Statistical Analysis:** Data were presented as percentages and frequency values for ordinal & categorical variables. Fisher's exact test was utilized to analyze the data. Along with the numerical data, we also included the means as well as the standard deviations. Shapiro-Wilk's gingivitis (I) was used as normality test. Paired t-tests were used for parametric data while signed rank test was used for numerical and ordinal non-parametric data. In each of the analyses, significance was determined by determining whether or not the p value was lower than 0.05. R Core Team's 2023 version 4.3.0 for Windows was utilized throughout every stage of

the statistical analysis process. R is a programming language & environment for statistical computing. Vienna, Austria is the location of the R Foundation for Statistical Computing. URL https://www.R-project.org/.)

## RESULTS

Demographic data: The study was done on 20 cases. There was 7 (35.0%) males & 13 (65.0%) females. The mean age was (33.05±6.56) years. Both sex and age are illustrated in table 1.

TABLE (1)

| Parameter | | | Value |
|---|---|---|---|
| **Gender** | **Male** | **N** | 7 |
| | | **%** | 35.0% |
| | **Female** | **N** | 13 |
| | | **%** | 65.0% |
| **Age** | **(Mean±SD) years** | | 33.05±6.56 |

The mean level of IL-34 in GCF in gingivitis cases was (172.10±17.11) & post 3 months from NSPT, it became (144.83±15.90). Table 2 shows a statistical significant difference among pre- & three months' post-operative period. ($p \leq 0.05$)

Table 2 shows that all clinical measurements showed a significant improvement.; PI was (2.55±0.51) and became (0.75±0.64) 3 months postoperatively, GI was (2.05±0.76) and became (0.60±0.50) 3 months postoperatively & PD was (2.80±0.41) and became (1.45±0.51) 3 months post-operatively. ($p \leq 0.05$)

According to demographic data, Spearman rank order correlation coefficient demonstrated that there was statistically insignificant correlation among IL-34 & both sex & age. ($p>0.05$). However, according to clinical parameters, Spearman rank order correlation coefficient demonstrated positive correlation among clinical parameters & IL-34 level in GCF which achieved statistical significance. ($p\leq0.05$) illustrated in table 3.

TABLE (2)

| (Mean±SD) | Baseline | 3months | P-value |
|---|---|---|---|
| **1- Plaque index (PI)** | 2.55±0.51 | 0.75±0.64 | **<0.001*** |
| **2- Gingival index (GI)** | 2.05±0.76 | 0.60±0.50 | **<0.001*** |
| **3- Probing depth (PD)** | 2.80±0.41 | 1.45±0.51 | **<0.001*** |
| **4- IL34 (ng/dl)** | 172.10±17.11 | 144.83±15.90 | **<0.001*** |

***; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)***

TABLE (3)

| | | | $r_s$ | p-value |
|---|---|---|---|---|
| **IL34 (ng/dl )** | **Clinical parameter** | **PI** | **0.517** | **<0.001*** |
| | | **GI** | **0.468** | **0.002*** |
| | | **PD** | **0.523** | **<0.001*** |
| | **Age** | | **-0.04** | **0.805ns** |
| | **Sex** | **Male** | | **0.142ns** |
| | | **Female** | | |

***$r_s$: Spearman rank order correlation coefficient *; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)***

According to questionnaire, results are illustrated in table 4

TABLE (4)

| Domain | Answer | Gingivitis (I) | |
|---|---|---|---|
| | | N | % |
| General Satisfaction | Strongly Agree | 9 | 45.0% |
| | Agree | 1 | 5.0% |
| | Uncertain | 0 | 0.0% |
| | Disagree | 2 | 10.0% |
| | Strongly Disagree | 8 | 40.0% |
| Technical Quality | Strongly Agree | 15 | 75% |
| | Agree | 5 | 25% |
| | Uncertain | 0 | 0% |
| | Disagree | 0 | 0% |
| | Strongly Disagree | 0 | 0% |
| Interpersonal Manner | Strongly Agree | 15 | 75.0% |
| | Agree | 5 | 25.0% |
| | Uncertain | 0 | 0.0% |
| | Disagree | 0 | 0.0% |
| | Strongly Disagree | 0 | 0.0% |
| Time Spent with Doctor | Strongly Agree | 10 | 50.0% |
| | Agree | 0 | 0.0% |
| | Uncertain | 5 | 25% |
| | Disagree | 4 | 20% |
| | Strongly Disagree | 1 | 5% |
| Communication | Strongly Agree | 0 | 0.0% |
| | Agree | 0 | 0.0% |
| | Uncertain | 0 | 0.0% |
| | Disagree | 1 | 5.0% |
| | Strongly Disagree | 19 | 95.0% |
| Accessibility and Convenience | Strongly Agree | 13 | 65% |
| | Agree | 4 | 20% |
| | Uncertain | 0 | 0.0% |
| | Disagree | 2 | 10% |
| | Strongly Disagree | 1 | 5% |

## DISCUSSION

Recently discovered cytokine, IL-34 which is another functional ligand as CSF-1 for the CSF-1R and can compensate the lack of CSF-1. Although both cytokines may have complimentary activities as they both activate the same receptor (CSF-1R) as detected in vivo studies and stimulate activation of macrophages and osteoclasts, they are temporally different as according to physiological action, IL-34 is more specific to certain tissues as neurons and epidermis while CSF-1 is widely spread. [17]

In pathogenic process, there is a relationship between IL-34 and CSF-1 as they are both detected in the fluid of inflamed joint & other inflammatory disorders, including rheumatoid arthritis.[18] Because of the similarity between the inflammatory processes of periodontal disease and rheumatoid arthritis, it was believed that the secretion of IL-34 by gingival fibroblasts and periodontal ligament cells via GCF plays a significant role in the pathogenesis of periodontal diseases.[19]

**Martinez et al. (2017)** detected the role of CSF-1 in gingivitis patients and found a direct correlation between the changes in the level of CSF-1 in saliva & clinical periodontal parameters in these patients. After NSPT,there was significant reduction in CSF-1 levels as well as clinical improvement. This correlation supported that CSF-1 play an important role in the pathogenesis of periodontal disease.[20] That's why IL-34 was suggested to have same role as CSF-1 in gingivitis patients.

This is the first clinical trial to detect the changes in IL-34 level in GCF in gingivitis cases after receiving NSPT. Therefore, the goal of this trial was to compare pre-NSPT IL- 34 levels in GCF to post-NSPT levels & to detect the correlation between these changes and changes in clinical periodontal parameters.

The mean IL-34 level in GCF has decreased significantly from its pre-treatment value of (172.10±17.11) to its post-treatment value of (144.83±15.90) after 3 months of NSPT. ($p \leq 0.05$)

These results suggested that IL-34 is a proinflammatory cytokine through action of TNF-α and IL-1β that regulate IL-34 expression by gingival fibroblast through NF-kB and Mitogen-activated protein kinase (MAPK) involved mechanisms.[21]

A study in 2018 showed results consistent with the results of this clinical trial as this study suggested that IL-34 could be classified as an inflammatory marker of periodontal disease because it was decreased in GCF after 8 weeks from NSPT in cases with generalized periodontitis.[22]

The results of the current research are also consistent with meta-analysis study that detected the effect of NSPT on proinflammatory cytokine levels, which showed that it reduced gingival inflammation and IL-6, IL-1, and TNF- levels, all of which influence IL-34 expression in GCF.[23] Because of the direct association between clinical parameters and GCF IL-34 levels, therefore, the decrease in GCF IL-34 levels was associated with significant improvement in all periodontal parameters ($p \leq 0.05$).

On the other side, Martinez et al. 2017 detected higher level of IL-34 in saliva of gingivitis & periodontitis patients in addition to negative statistical correlation with the clinical periodontal parameters.[20] Another study by Lira-Junior et al., 2021 concluded that salivary IL-34 was significantly lower in cases with periodontal disease at baseline, but higher level was detected 3 months following NSPT when compared to baseline levels.[24]

There were inconsistencies between the results of previous two studies and this current clinical trial which might be due to differences in sample size and the identification of biomarkers in different diagnostic media.

Important limitations were detected in this trial such as the extremely wide range of age and that the Periotron apparatus was not utilized for GCF volume calculation. In addition, neither healthy nor diseased cases of periodontal inflammation were included in the study. Additional limitations may include the limited sample size, as well as the requirement for longer follow-ups in larger cohort study in order to figure out whether or not this marker may be able to predict disease activity.

## CONCLUSION

According to the findings of the current clinical trial, the conclusion is that NSPT leads to reduction in GCF IL-34 level. As a consequence of this, IL-34 has the potential to be beneficial as both a diagnostic marker and as a potential target for periodontal disease therapy in the future.

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